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## Metabolic parameters as indicators of broiler chicken welfare and meat qualify

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## CHAPTER 3

### **Electrical stunning and exsanguination decrease the extracellular volume in the broiler brain, as studied with brain impedance recordings**

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## ABSTRACT

Electrical stunning in the process of slaughtering poultry is used to induce unconsciousness and to immobilize the animal for easier processing. Unconsciousness is a function of brain damage. Brain damage has been studied with brain impedance recordings under ischemic conditions. This experiment studies brain impedance as a response to a general epileptiform insult caused by electrical stunning and ischemia caused by exsanguination. Brain impedance was recorded in 10 broiler chickens for each of three killing methods: whole body electrical stunning, which induces cardiac arrest; head only electrical stunning followed by exsanguination; and exsanguination without stunning. Brain impedance was converted into relative extracellular volume (ECV) values. Results showed that, immediately after electrical stunning, the ECV decreased 5.5% from base ECV. With exsanguination only, the ECV decreased from base ECV only after 4 min after neck cutting. The ECV decrease after 10 min did not differ between treatments. With a time of 228 s. to reach one-half the ECV decrease found at 10 min, electrical stunning resulted in much faster change in ECV than exsanguination only (373 s). Within the head only stunning group, six animals showed a response similar to that found with whole body stunning; the other four animals responded like the animals that were exsanguinated only. It was concluded that brain impedance recordings used with electrical stunning reflect brain damage. This damage was both epileptic and ischemic in nature. Whole body stunning induced immediate brain damage, suggesting an adequate stun was delivered. The dual response found with head only stunning might indicate that this stunning method does not always give an adequate stun.

**Key words:** electrical stunning - exsanguination - brain impedance recordings - extracellular volume - unconsciousness

## **INTRODUCTION**

In the process of slaughtering poultry, electrical stunning is used both to make the slaughter procedure as humane as possible, by rendering the animals unconscious, and to immobilize them for easier killing. In practice, however, electrical stunning in high rate slaughter lines does not stun all chickens adequately (Heath, 1984); thus, chickens may suffer during death struggle. With regard to the final product, inadequate stunning can lead to an increased number of red skin carcasses, blood spots in the meat, and color differences (Griffiths, 1985; Hillebrand et al., 1996). The final result of inadequate stunning is increased animal suffering and poorer meat quality (Veerkamp and De Vries, 1983; Gregory and Wotton, 1987a; Gregory and Wilkins, 1989; Schütt-Abraham, 1995). This study will focus on the aspect of unconsciousness only.

Although it is impossible to measure unconsciousness as such, certain pathological states of the brain indicate a state of unconsciousness. One of these states which is of importance with electrical stunning is a general epileptiform insult (Hoenderken, 1978). The main method used to record epileptiform insults is electroencephalography (EEG), which measures the electrical activity of the brain. In humans, the EEG is a reliable indicator of unconsciousness during a general epileptiform insult (Hoenderken, 1978; Lopes da Silva, 1983), but not so in poultry. Poultry exhibit a different EEG pattern, which in part resembles a different epileptic condition in humans. Such a condition is not a general epileptiform insult and not all people lose consciousness during it (Gregory and Wotton, 1987b; Raj, 1998).

An alternative approach for determining unconsciousness is to record brain impedance. Impedance of the living brain is determined by the size of the extracellular space in the tissue (Van Harreveld, 1966). This space, which is of constant volume under normal conditions, constitutes about 20 to 30% of the whole brain volume in rats. Unpublished results indicate this is about 50% in broiler chickens. This space is conductive for electric current, as intact cells are electrically inert (Van Harreveld, 1972). When the brain becomes metabolically compromised, energy-requiring processes, such as the  $\text{Na}^+/\text{K}^+$  pump in the cell membrane, fail, and cells can no longer maintain the ion gradients over the outer membrane. Ion gradients shift, and extracellular water enters the brain cells, thus leading to a decreased extracellular volume (ECV) and an increase in tissue impedance. In this state of the brain, when the outer cell membranes have not yet disintegrated, neurons can no longer function (Klein et al., 1989). Such a state usually develops after the EEG becomes iso-electric.

Metabolic brain function has been a good indicator of brain damage (Van Harreveld, 1966). The onset and development of brain damage as expressed by change in the ECV has been studied in numerous animal species, including poultry. In these studies, brain impedance recordings were used to study brain damage caused by ischemia. The ECV decreased within 1 min after cardiac arrest

### Chapter 3

was induced and levelled at approximately 55% of its original size after 10 min (Van Harreveld, 1972; Korf et al., 1988; Ruis-Heutink et al., 1998). However, no research has been done using brain impedance recordings to study brain damage caused by an epileptiform insult, as caused by the electrical stunning of poultry during slaughter.

The purpose of this study was to determine how brain impedance, as a measure of brain damage in broiler chickens, would be affected by electrical stunning and exsanguination. Brain impedance was recorded using two stunning methods, whole body electrical stunning and head only electrical stunning, and was compared with the results from animals that were exsanguinated without prior stunning. In this way, the effects of an epileptiform insult and progressive ischemia could be studied, and brain impedance recordings could be evaluated as an alternative to EEG recordings.

## MATERIALS AND METHODS

### Animals and housing

Thirty commercially reared Cobb broiler chickens of about 2 kg body weight were purchased from a commercial slaughter plant. Chickens from a commercial strain were chosen for practical relevance. The chickens were transported to the experimental animal facility of the Institute for Animal Science and Health and housed under commercial conditions (group housed on litter at 20 °C, 23 to 1 h light to dark regime, feed and water available ad libitum). The chickens were allowed 24 h of rest after transport. Approval for carrying out the experiment was obtained from the ethical committee of the Institute for Animal Science and Health<sup>1</sup>.

Eight hours before surgery, feed was removed from the pen. Anaesthesia was administered by an i.m. injection of 2 mL Ketamine<sup>2</sup>, followed 15 min later by an i.v. injection of 0.3 to 0.8 mL Nembutal<sup>3</sup>. The chickens were equipped with a pair of silver electrodes in the striatum area of the brain as described by Ruis-Heutink et al. (1998). The chickens were then allowed 24 h to recover.

### Treatments

On the testing day the chickens were hung by the legs from shackles, and the electrodes were connected to an impedance recording device<sup>4</sup>. Base impedance

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signal was recorded for 5 min. With each of the following three stunning and killing methods 10 chickens were killed.

- Whole body electrical stunning at 220 V for 4 s. With one electrode on the comb and the other on the cloaca, whole body stunning was chosen to simulate the passage of electric current through the head and body as would happen when using a water bath stunner. The stun was set at 220 V, which is higher than is being used in commercial slaughter plants, in order to guarantee an epileptiform insult and cardiac fibrillation in all birds.
- Head only electrical stunning at 100V for 4 s immediately followed by exsanguination by cutting one jugular vein. Head only stunning was applied by a pair of scissor-like tongs that made contact with both sides of the head. This method was studied as an alternative to whole body stunning. The stun was set at 100V, and the current passed directly through the brain.
- Exsanguination only by cutting one jugular vein; no prior stunning. These animals were not stunned so that the purely ischemic effect of the slaughter process could be studied.

In each chicken, brain impedance was recorded for 10 min, as described by Ruis-Heutinck et al. (1998), after stunning and bleeding. The experiment was carried out over 5 d, measuring brain impedance in six animals per day. Treatments were assigned at random throughout these days.

### **Histological analysis**

After the impedance recordings were concluded, the brain was dissected from the skull and stored in a 4% paraformaldehyde solution for at least 2 wk. The actual electrode positions were determined by comparing 40 µm thick brain slices with a stereotaxic atlas (Kuenzel and Masson, 1988) as described by Ruis-Heutinck et al. (1998).

### **Presentation of results and statistics**

For each chicken the ECV in the striatum was calculated according to Maxwell's equation:

$$\frac{ECV_i}{ECV_B} = \frac{R_B}{R_i}$$

with  $ECV_B$  is the base ECV before stunning or neck cutting (defined as the 100% value),  $R_B$  is the recorded tissue impedance before stunning or neck cutting, and  $ECV_i$  and  $R_i$  the ECV and recorded tissue impedance at time  $i$  minutes after stunning or neck cutting, respectively. Thus,  $ECV_i$  was thus expressed as the percent deviation from the base ECV. The delay time until the ECV differed

### Chapter 3

significantly from the base level is expressed in seconds after stunning or neck cutting. For the dependent variables  $y$ , change in ECV at time 0 (immediately after stunning or neck cutting) and time 10 min after stunning or neck cutting, and time needed to reach 50% of the change in ECV at 10 min, analysis of variance was run using  $y_{ij} = SKM_i + e_{ij}$  as a model;  $SKM_i$  was one of the three stunning and killing methods. Pairwise comparisons between ECV at different times was done by means of Student's  $t$ -test. Data are represented as mean  $\pm$  s.e. The Genstat statistical software package was used for all tests (Genstat 5, 1993).

## RESULTS

### Whole body electrical stunning, inducing cardiac fibrillation

In 10 broiler chickens, brain impedance was recorded for 10 min following whole body electrical stunning. For each animal, the ECV was calculated from the impedance data at 1-min intervals. The ECV response patterns for each stunning and killing method are shown in figure 1. ECV at 10 min after stunning was significantly ( $P < 0.05$ ) decreased from base ECV before stunning, which was defined as the 100% value within each individual chicken ( $\Delta ECV_{Base-10} = 21.6 \pm 2.8\%$ ). The ECV was significantly ( $P < 0.05$ ) decreased immediately after stunning ( $t = 0$ ) compared to base ECV ( $\Delta ECV_{Base-0} = 4.4 \pm 0.8\%$ ). The time needed to reach 50% of the decrease in ECV found at 10 min after stunning was used as a characteristic for the rate of decrease ( $t_{0-50\%} = 201 \pm 15$  sec.).

### Exsanguination without prior stunning

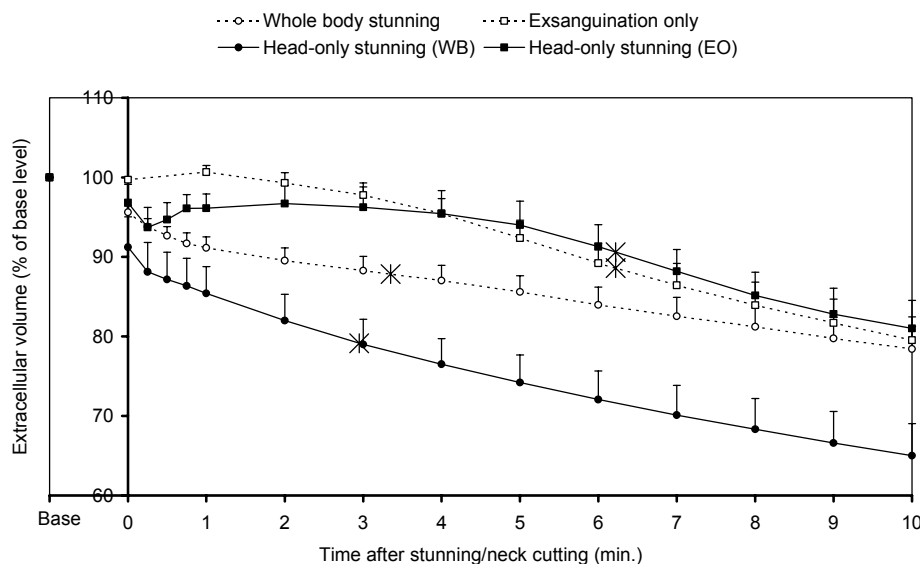
In 10 broiler chickens, brain impedance was recorded for 10 min following exsanguination without prior stunning. The ECV was calculated from the impedance data at 1-min intervals. ECV at 10 min after neck cutting was significantly ( $P < 0.05$ ) decreased from base ECV ( $\Delta ECV_{Base-10} = 28.6 \pm 3.7\%$ ). The ECV showed an initial delay before it decreased significantly from base ECV, which first occurred at 4 min after neck cutting. The time needed to reach 50% of the change in ECV at 10 min was  $373 \pm 19$  s.

### Head only electrical stunning followed by exsanguination

In 10 broiler chickens, brain impedance was recorded for 10 min following head-only electrical stunning and neck cutting. In contrast with the consistent responses found in the groups within each of the other treatments, in the group of chickens that received head only stunning, two separate patterns in ECV response could be distinguished. The distinction was made on the basis of the ECV at time 0 and of the time needed until the ECV first differed significantly from base ECV. The first pattern ( $n = 6$ ) resembled the response pattern of animals in the whole body

### Extracellular volume responses with various killing methods

stunning group in that it showed a significant ( $P < 0.05$ ) decrease in ECV immediately after stunning ( $\Delta ECV_{\text{Base-0}} = 8.8 \pm 3.8\%$ ) and a continuing but gradually levelling decrease in ECV. The decrease in ECV at 10 min after stunning was  $35.0 \pm 4.0\%$ . The time needed to reach 50% of this change was  $= 177 \pm 16$  s. The second pattern ( $n = 4$ ) resembled the response pattern found in the exsanguination only group and showed a delay of 5 min before the ECV differed significantly ( $P < 0.05$ ) from base ECV. The decrease in ECV at 10 min after stunning was  $19.0 \pm 3.5\%$ . The time needed to reach 50% of this change was  $373 \pm 41$  s.



**Figure 1.** Change in extracellular volume following whole body electrical stunning (220V, 4s) inducing cardiac arrest ( $n = 10$ ), exsanguination only (without prior stunning,  $n = 10$ ), and head only electrical stunning (100V, 4s). The head only stunning responses are split into a subgroup that reacts similar to the whole body electrical stunning group (WB,  $n = 6$ ) and a subgroup that reacts like the exsanguination only group (EO,  $n = 4$ ). Base ECV before stunning is defined as 100%. Data are given as mean  $\pm$  s.e. Time needed to reach 50% of the change in extracellular volume at 10 min. is indicated with a \* marker on each line.

### Comparison of stunning/killing methods

The decrease in ECV at 10 min after stunning or neck cutting did not differ between stunning and killing methods, except for the subgroup of the head only stunning group, which showed a response similar to the whole body stunning group [a significantly ( $P < 0.05$ ) greater decrease]. The immediate ( $t = 0$ ) decrease in ECV after stunning did not differ between the this subgroup and the whole body



### Chapter 3

stunned group, but both were significantly ( $P < 0.05$ ) greater than in the exsanguination only group. Time needed until 50% of the ECV change at 10 min was reached was significantly lower ( $P < 0.05$ ) in the whole body stunned group and the subgroup of the head only stunned group that responded in a similar way when compared to the exsanguination only group and the similarly responding head only stunned subgroup.

#### **Histological examination of brain tissue**

Histological examination of the brain tissue samples confirmed that all electrodes were indeed located in either the hyperstriatum ventrale or in the neostriatum. This confirms that the stereotaxic coordinates used in the surgical procedure were correct.

## **DISCUSSION**

Whole body electrical stunning, which was previously reported to induce cardiac fibrillation using this voltage (Gregory and Wotton, 1987b; Gregory, et al., 1991), and head only stunning and exsanguination that results in a similar brain impedance pattern as whole body stunning, cause both epileptic and ischemic conditions in the brain. These conditions cause immediate and progressive brain damage in broiler chickens at the voltages used in this experiment. An epileptiform insult is generated; this immediately depolarizes all affected neurons, and the stimulated glycolysis reduces cellular energy reserves (Kuhr, et al., 1988). Lack of supply of oxygen and nutrients continues under ischemic conditions and causes a decrease of the extracellular space and increase of the intracellular volume of the brain (Korf, et al., 1988). These changes are independent of the blood volume in the brain, which is only about 2% of the total brain volume. Under these circumstances, the neuron is no longer functional. Continuation of this state will cause the brain damage to become irreversible. Brain impedance recordings after electrical stunning and exsanguination were demonstrated to reflect change in ECV in this experiment.

With whole body electrical stunning, brain impedance increased instantaneously and progressively in all animals. The instantaneous increase indicates that immediate brain damage is established. Such an increase would be caused by a compromised cellular metabolism, leading to net fluxes of cations ( $\text{Na}^+$ ,  $\text{K}^+$ ) over the outer cell membrane. Cellular metabolism failing this way indicates severe brain dysfunction, and therefore we may assume that there is an instantaneous and lasting unconsciousness (Lopes da Silva, 1983).

In this model, a 220 V electrical stun was used, which is higher than the voltages used under practical slaughter conditions (Heath et al., 1994). This

current was chosen to guarantee the occurrence of cardiac fibrillation (Gregory and Wotton, 1987b). However, a 220 V electrical stun may also cause more severe brain damage by itself than the currents used in practice (Reilly, 1994). The instantaneous decrease of the ECV in the brain supports the EEG studies regarding the quality of whole body methods of electrical stunning for loss of consciousness, given a sufficiently high current and proper application of the stun (Gregory and Wotton, 1987b).

Exsanguination without prior stunning was used as a treatment in which brain damage was entirely ischemic, without interference from electrically induced effects. Brain impedance showed a delay before beginning to increase, which suggests that conscious chickens have a mechanism to keep the energy metabolism and blood supply to the brain at an adequate level for some time. This delay could be explained by the fact that the cut was made in the jugular vein only, whereas both carotid arteries and, thereby, the afferent tract to the brain remained intact. Also, chickens have a strong capacity to mobilize tissue fluid to compensate for low blood pressure (Hillman and Lundvall, 1981). Exsanguination without prior stunning results in a less increased brain impedance over 10 min than the induction of cardiac arrest without exsanguination (Ruis-Heutink et al., 1998), which may be due to the mechanisms mentioned previously. The change in impedance established at 10 min after neck cutting is comparable with the change in impedance found at 10 min after electrical stunning. Under slaughter conditions, brain damage must necessarily become irreversible, and the animal will not regain consciousness.

On average, head only stunning followed by exsanguination did not result in a different brain impedance response than whole body stunning. However, the response patterns for specific birds after head only stunning could be divided into two groups: one resembling the pattern found after whole body stunning, in that it showed an immediate and ongoing, but gradually levelling decrease in ECV, and the other similar to the pattern found after exsanguination only, in that it showed a delay of several minutes before decreasing, followed by a fairly steep decrease which gradually levels off. The cause of this difference in response patterns is not clear. One possible cause could be that the applied voltage of 100 V for head only stunning is a threshold value or range for the induction of immediate brain damage, even though EEG studies showed the occurrence of an epileptiform insult with as little as 27 V head only stunning (Hillebrand et al., 1993). If this is the case, head-only stunning would then require a higher voltage to deliver an effective stun than is issued with the conventional water bath stunner now used (Gregory and Wotton, 1987a). Another possible cause could be that the broiler chickens are physiologically still chicks, whose immature brain might react differently to the impact of electrical current than the brain of adult chickens (Izard, 1980). Therefore, a variation in the stage of brain development could contribute this effect. Although the effect of stress on the effectiveness of electrical stunning has not been studied, pre-slaughter handling and shackling are stressful events and such

### Chapter 3

stressors might affect the parameters associated with either stunning method. The fact that the subgroup responding like the whole body stunned group showed a larger decrease in ECV than the whole body stunned group itself might be explained by a stronger current running through the brain because of the positions of the electrodes. Amperage measurements between the electrodes do not determine the amount of current passed through the brain, and no other method is currently available to determine electrical current pathways through various parts of the body during electrical stunning.

Concluding, brain impedance can be used as a parameter to determine the onset and development of brain damaged reflected by a reduction in ECV in the brain. With proper application and a sufficiently high voltage, preferably inducing cardiac fibrillation, the immediate and lasting decrease in ECV indicates that whole body electrical stunning is an efficient method to induce immediate unconsciousness that lasts until the death of the animal. This effect seems both epileptic and ischemic in nature. Based on brain impedance recordings only, at 100 V, head only stunning does not provide a sufficiently reliable alternative to whole body stunning. The reasons for a lack of consistency are not clear. Measuring other parameters or developing methods to determine the flow of the stunning current through the body could provide answers to this problem and suggestions for criteria required to deliver a proper stun using the head only method.

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*Extracellular volume responses with various killing methods*

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### *Chapter 3*

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